

Understanding the immunoangiostatic CXC chemokine network

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Chemokines, originally discovered as mediators of directional migration of immune cells to sites of inflammation and injury, have a function beyond their role in leukocyte chemotaxis. Indeed, they participate in organ development, angiogenesis, tumourigenesis and, more importantly, in the immune response. The chemokine family characterized by four highly conserved cysteine amino acid residues, with two cysteine residues (C) and a non-cysteine amino acid (X) between them (CXC), is known for its ability to promote trafficking of various leukocytes and to regulate angiogenesis and vascular remodelling. Intriguingly, the presence or absence of a structural-functional domain constituted by glutamic acid-leucine-arginine motif that precedes the first cysteine amino acid residue accounts for their unique property to induce or inhibit angiogenesis (angiogenic or angiostatic activity). The ability of CXC chemokine receptor 3 to promote Th1-dependent immunity and, at the same time, inhibit angiogenesis (immunoangiostasis) is of critical importance for inducing tumour regression. Agents that are able to inhibit angiogenic activities or promote angiostatic activities of CXC chemokines are future targets for research on cancer treatment. Here, we review insights on CXC chemokines in the context of immunoangiostasis and vascular damage.

1. Introduction

Angiostasis is the strict restraint over creation of new blood vessels, which is the normal state (homeostasis) for adult humans.^{1,2} Angiogenesis is the opposite condition as it involves the generation of new blood vessels, occurring in a number of physiological and pathological processes such as embryonic development, wound healing, chronic inflammation, and the growth of malignant solid tumours.³ During wound repair, resting endothelial cells undergo activation that leads to matrix proteolysis, migration, proliferation, and development of new capillaries in a strictly controlled and transient way, depending on the levels of angiogenic and angiostatic mediators expressed in the wounded tissue.⁴ The rate of normal capillary endothelial cell turnover in adults is typically measurable in months or years.⁵

In contrast to the precise regulation of wound-associated angiogenesis, an imbalance between angiogenic factors and angiostatic substances determines tumour angiogenesis.^{6–8}

Recently, the biology of chemokines has extended beyond their role in mediating leukocyte trafficking. In particular, CXC chemokines have been found to be important in the regulation of angiogenesis, angiostasis, and in promoting tumour cell migration and organ-specific metastases.^{9–12} Indeed, cytokines produced by tumours and chemokines regulate neoplastic outcome by directing angiostasis or angiogenesis (Figure 1). Angiogenesis is the result of an imbalance in the over-abundance of angiogenic factors compared with relative under-expression of angiostatic factors. Abundant production of pro-inflammatory cytokines can lead to a level of inflammation that potentiates angiogenesis, thus favouring neoplastic growth. Alternatively, high levels of monocytes and/or neutrophil infiltration, in response to an altered balance of pro- vs. anti-inflammatory cytokines, can be associated with cytotoxicity, angiostasis, and possible tumour regression.¹³ In particular, in tumours, interleukin-10 (IL-10) is generally a product of tumour cells and tumour-associated macrophages.¹³ Profiling of tumour-associated macrophages from a murine sarcoma revealed unexpected expression of interferon-inducible chemokines associated with a high expression of IL-10 and a low expression of IL-12.¹⁴

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Chemokines are small proteins, 70–120 residues long, that contain 1–3 (usually 2) disulfides. Their sequence homology is highly variable, but they all share very similar secondary and tertiary structures with 20–40% sequence identity across the whole superfamily. However, the quaternary structure is dramatically different between subfamilies. Their classification includes four structural branches (C, CC, CXC, and CX3C) according to the number and the spacing of the first two cysteine residues in the amino-terminal part of the protein. To date, 47 chemokines and 20 chemokine receptors have been identified in humans, but this number might not take into account all described variants that can increase their effective complexity.^{15,16}

The CXC chemokines, a pleiotropic family of cytokines, promote the trafficking of various leukocytes and regulate angiogenesis, particularly in cardiovascular disease, by contributing to the homing of haematopoietic progenitors, B-lymphocyte development, and progenitor recruitment to sites of ischaemic tissue damage.⁵ Members of the CXC family are characterized by a pair of cysteine residues separated by a single non-cysteine residue, represented by the letter X. It includes 16 ligands and eight receptors in humans, as many different ligands bind the same receptors or multiple receptors. This redundancy may allow chemokine/chemokine receptor pairs to play an exceptional fine-tuning role for the immune system.⁹ The CXC chemokine structure consists of a disordered N-terminus of 6–10 amino acids followed by a long N-loop, with a three-stranded anti-parallel β -sheet that ends in a 3_{10} C-terminal helix; the N terminus functions as a key signaling domain, while the N-loop contains important binding determinants¹⁷ (Figure 2). Many CXC chemokines also form dimers, higher order homo-oligomers and hetero-oligomers that have been proven to affect chemical function.^{18,19} Indeed, oligomerization appears to be associated with glycosaminoglycan (GAG) binding, which is an essential interaction for some chemokines, and, notably, mutations in the GAG binding sites of three chemokines—CCL2, CCL4, and CCL5—affected their ability to recruit cells *in vivo*.¹⁹ These mutant chemokines retained chemotactic activity *in vitro*, but they were unable to recruit cells when administered intraperitoneally. Additionally, monomeric variants, although fully active *in vitro*, were devoid of activity *in vivo*, demonstrating that both GAG binding and the ability to form higher-order oligomers are essential for the activity of particular chemokines *in vivo*, although they are not required for receptor activation *in vitro*.¹⁹ The CXC family can be further subdivided into two categories depending on the presence or absence of the so-called 'ELR motif' (the sequence Glu-Leu-Arg), which immediately precedes the first cysteine residue near the amino-terminal end and is critical for receptor binding and essential for the chemotactic activity.^{20,21} Depending on the presence or absence of the ELR motif in their amino-terminal end, CXC chemokines are potent promoters or inhibitors of angiogenesis.^{5,20,22}

The chemokine receptors are a subset of G protein-coupled receptors with seven helical membrane-spanning regions connected by extramembranous loops. Several CXC chemokine receptors that participate in the complex networks of tumour angiogenesis and angiostasis have been identified.²² Among these, CXC chemokine receptor 3 (CXCR3) receptors and their ligands are major chemoattractants for the recruitment of Th1 cells expressing CXCR3

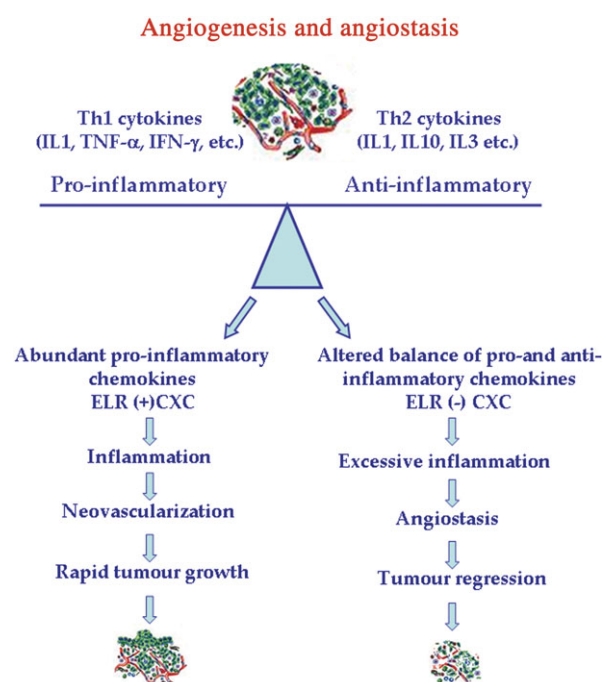


Figure 1 Opposing states of angiogenesis and angiostasis. The balance of cytokines in tumours is critical for regulating the type and the extent of inflammatory infiltration. Cytokines produced by tumours and chemokines regulate neoplastic outcome by directing angiostasis or angiogenesis. Angiogenesis is the result of an imbalance in the over-abundance of angiogenic factors compared with relative under-expression of angiostatic factors that determines rapid tumour growth. Angiostasis is associated with high levels of monocyte and/or neutrophil infiltration in response to an altered balance of pro-inflammatory vs. anti-inflammatory cytokines.¹³

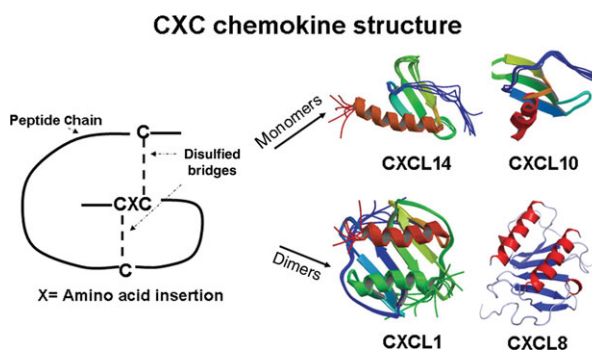


Figure 2 CXC chemokine structure. Examples of ribbon diagram structures of chemokines solved by X-ray crystallography as monomers (CXCL10, 14) and dimers (CXCL1, 8). The general structure of CXC chemokine monomers (CXCL10, 14) consists of a three-stranded anti-parallel β -sheet that ends in a C-terminal helix (red). Many CXC chemokines also form dimers, higher-order homo-oligomers, and hetero-oligomers. Dimer structures (CXCL1, 8) consist of a six-stranded antiparallel β -sheet packed against two C-terminal antiparallel α -helices.

during cell-mediated immunity.²² Here, we review the current knowledge on the properties of the CXC chemokines, focusing on immunoangiostasis.

2. Angiogenic CXC chemokines

CXC chemokines containing the ELR motif (ELR+) specifically recruit neutrophil polymorphonuclear leukocytes into inflamed tissues in a multi-step process of rolling, adhesion,

and transmigration.^{23,24} Characteristic of ELR+ chemokines is their ability to promote angiogenesis.^{22,25} Specifically, CXCL8, CXCL5, and CXCL1, 2, 3 activate neovascularization in rabbit cornea and have chemotactic effects on endothelial cells *in vitro*.²⁶ In contrast, CXC chemokines lacking the ELR motif (ELR-), such as CXCL4, CXCL9, and CXCL10, inhibit the angiogenesis induced by ELR+ CXC chemokines.^{22,25}

The role of CXC chemokines in angiogenesis and angiostasis may be affected by multiple factors, and the structurally-based classification of CXC chemokines has some exceptions. Indeed, CXCL12, an ELR- CXC chemokine, also known as stromal cell-derived factor-1 α , has a demonstrated angiogenic activity both *in vitro* and *in vivo*;²⁷⁻²⁹ its receptor is CXCR4, whereas all the other ELR+ CXC act via CXCR1 and CXCR2. The role of CXCR4 in tumour angiogenesis has now been established.³⁰ Several studies have shown that CXCR4 promotes tumour progression by direct and indirect mechanisms, and its influence on tumourigenesis is due to its angiogenic properties, observed also during tissue ischaemia and vascular repair.^{31,32} Indeed, CXCR4 is essential for metastatic spread to organs where CXCL12 is expressed, and thereby allows tumour cells to access cellular niches, such as the marrow, that favour tumour cell survival and growth. Moreover, stromal-derived CXCL12 itself can stimulate survival and growth of neoplastic cells in a paracrine fashion and can promote tumour angiogenesis by attracting endothelial cells to the tumour microenvironment.³² In human kidney cancer cell lines and in patient specimens, CXCL12 is not expressed although its receptor is functionally active.³³ In other tumour specimens, CXCR4 was shown to be present, but it did not promote tumour-associated angiogenesis. Moreover, the neutralization of CXCR4 and its ligand CXCL12 significantly inhibited *in vivo* metastasis, but did not appear to significantly reduce the size of the primary tumour.^{34,35} Collectively, these observations reveal that CXCR4 is an important molecule involved in the spread and progression of a variety of different tumours.

More recently, strong evidence showed the involvement of CXCR4/CXCL12 in the metastasis of rhabdomyosarcoma, delineating a novel basis for future studies on the mechanisms of metastasis.³⁶ Moreover, high expression of nuclear CXCR4 was significantly correlated with lymph node metastasis in breast cancer.³⁷ The high expression of nuclear CXCR4 in hormone receptor-negative breast cancer was associated with a high possibility of lymph node metastasis. In addition, CXCR4 receptors contribute to renal cell carcinoma dissemination and may provide a novel link between CXCR4 chemokine receptor expression and integrin-triggered renal cell carcinoma adhesion to the vascular wall and subendothelial matrix components.³⁸ Interestingly, 15-deoxy- δ (12,14)-prostaglandin J(2) has been shown to down-regulate CXCR4 on cancer cells through both peroxisome proliferator-activated receptor γ and nuclear factor- κ B and impact upon the overall process of tumour expansion.³⁹ Thus, the interest in the field of CXCR4 involvement in metastasis is increasing. Among the chemokine subfamilies (C, CC, or CX3C), CCL1, CCL2, CCL11, and CX3CL1 show angiogenic properties.

Elevated ELR+ CXC chemokine production, initially driven by inflammatory stimuli and later by hypoxia of the tumour microenvironment, regulates angiogenesis⁴⁰ and, therefore, tumour progression. The ELR+ CXC chemokine CXCL8 has

been associated with neovascularization and tumourigenesis of ovarian carcinoma.⁴¹ A human lung squamous carcinoma cell line that produces little CXCL8 but abundant CXCL10 did not grow in severe combined immunodeficiency mice.⁴² In contrast to tumour cells producing CXCL8, treatment of mice bearing CXCL8-producing tumours with anti-CXCL8 antibodies or with angiostatic chemokine CXCL10 inhibited tumour growth and metastasis.^{42,43} It has also been demonstrated that CXC chemokines play a significant role in the development and growth of prostate tumours⁴⁴ and renal carcinoma.⁴⁵ Moreover, elevated levels of CXCL5 and CXCL8 were detected in non-small cell lung cancer and correlated with the vascularity of these tumours.⁵ Most of the results concerning the activity of chemokines in angiogenesis and angiostasis were obtained from *in vitro* or specifically designed experiments in animals. More precise information should probably be obtained from knockout models. These results triggered the idea of the therapeutic use of selected chemokines or their inhibitors to manipulate angiogenesis in tumour and wound healing. Mice lacking CXCL12 or its receptor CXCR4 exhibit defects in cardiovascular development, providing clear evidence of the important role of this chemokine/receptor interaction in angiogenesis during development.^{46,47}

3. Angiostatic CXC chemokines

The interferon-inducible ELR- CXC chemokine members of the CXC chemokine family inhibit endothelial cell proliferation, chemotaxis, activation of Th1 cells, natural killer (NK) cells, macrophages, dendritic cells (DC), and hematopoiesis. They are known to act as angiostatic agents, thus inhibiting tumour growth.²⁰ ELR- CXC chemokines are listed in Table 1.^{22,25}

The angiostatic chemokines CXCL9, CXCL10, and CXCL11 are interferon-inducible ELR- CXC chemokines with potent inhibitory properties towards angiogenesis, and their biological function in the inhibition of angiogenesis is linked to cell-mediated immunity. They also act in response to several angiogenic factors, including ELR+ CXC chemokines, basic fibroblast growth factor (bFGF), and vascular endothelial growth factor (VEGF).⁵

This relationship between interferon and interferon-inducible CXC chemokines and their biological function is critically relevant to the functional activity of other cytokines such as Th1/type 1 cytokines.^{48,49} Cytokines such as IL-23, IL-18, IL-15, IL-12, and IL-2 have profound effects on the production of CXCL9, CXCL10, and CXCL11 via the induction of interferon- γ and induce a chemokine gradient (Figure 3). Furthermore, the cytokine cascade is connected with Th1/type1 cytokine-mediated immunity, which involves NK, T lymphocytes expressing CD4 (CD4 cells), and CD8 surface protein (CD8 cells), towards tumour-associated antigens. The appropriate immune response to tumour-associated antigens and the subsequent generation of angiostatic factors that can further modify local tumour-associated angiogenesis has led to the concept of immunoangiostasis.¹² This concept contains the complex notion of a combined activation of mononuclear cells expressing interferon-inducible ELR- CXC receptors and, at the same time, the enhancement of interferon-inducible ELR- CXC production that will promote a greater mononuclear cell extravasation within the tumour, inducing enhanced type 1

Table 1 Human chemokines and chemokine receptors

Systematic name	Synonyms	H/I	Major target cell showing chemotaxis	Specific receptor
CXC Chemokines				
CXCL1	GRO α , MGSA- α , NAP-3	I	Neutrophils, endothelial cells	CXCR1,2
CXCL2	GRO β , MIP-2 α , MGSA- β , CINC-2 α	I	Neutrophils, endothelial cells	CXCR2
CXCL3	GRO γ , MIP-2 α , CINC-2 β	I	Neutrophils	CXCR2
CXCL5	ENA-78	I	Neutrophils	CXCR2
CXCL6	GCP-2	I	Neutrophils	CXCR1,2
CXCL7	LDGF-PBP, CTAPIII, NAP-2, LA-PF4, MDGF, LDGF, β -TG	I	Fibroblasts	CXCR1,2
CXCL8	IL-8, NAP-1, MDNCF, GCP-1	I	Neutrophils, T cells, basophils, endothelial cells	CXCR1,2
CXCL4	PF4	U	Fibroblasts, endothelial cells	CXCR3A,3B ^a
CXCL4V1		U	Fibroblasts, endothelial cells	CXCR3A,3B
CXCL9	MIG	I	T cells, progenitors	CXCR3B
CXCL10	IP-10, CRG-2	I	T cells	CXCR3B
CXCL11	I-TAC, β -R1, IP-9	I	T cells	CXCR3B
CXCL12	SDF-1 α / β , PBSF	H	Monocytes, B cells, haematopoietic progenitors, non-haematopoietic cells	CXCR4,7
CXCL13	BCA-1, BLC	H	T cells, naïve B cells	CXCR5
CXCL14	BRAK, Bolekine	I		Unknown
CXCL16	SRPSOX	I	T cells, NK cells	CXCR6
CXCL17	DMC, VCC-1	U		Unknown
CC Chemokines				
CCL1	I-309, TCA3, P500	I	Monocytes, T cells	CCR8
CCL2	MCP-1, MCAF	I	Monocytes, T cells, basophils, NK cells, progenitors	CCR2
CCL3	LD78 α , MIP-1 α	I	Monocytes, T cells, NK cells, basophils, eosinophils, dendritic cells, haematopoietic progenitors	CCR1,5
CCL3L1	LD78 β	I	Monocytes, T cells, NK cells, basophils, eosinophils, dendritic cells, haematopoietic progenitors	CCR1,5
CCL3L3	LD78 β	I	Monocytes, T cells, NK cells, basophils, eosinophils, dendritic cells, haematopoietic progenitors	CCR1,5
CCL4	MIP-1 β , Act-2, G-26, HC21, H400, LAG-1, SIS γ , MAD-5	I	Monocytes, T cells, dendritic cells, NK cells, progenitors	CCR5
CCL4L1	At744.2	I	Monocytes, T cells, dendritic cells, NK cells, progenitors	CCR5
CCL4L2		I	Monocytes, T cells, dendritic cells, NK cells, progenitors	CCR5
CCL5	RANTES	I	T cells, eosinophils, basophils, NK cells, dendritic cells	CCR1,3,5
CCL7	MCP-3	I	Monocytes, T cells, eosinophils, basophils, NK cells, dendritic cells	CCR1,2,3
CCL8	MCP-2, HC14	I	Monocytes, T cells, eosinophils, basophils, NK cells	CCR1,2,3,5
CCL11	Eotaxin	I	Eosinophils, T cells	CCR3,5
CCL13	MCP-4, NCC-1, CK β 10	I	Monocytes, T cells, eosinophils	CCR1,2,3
CCL14	HCC-1, HCC-3, NCC-2, CK β 1, MCIF	H	Monocytes, haematopoietic progenitors	CCR1,5
CCL15	HCC-2, NCC-3, MIP-5, Lkn-1, MIP-1	H	Monocytes, T cells, eosinophils	CCR1,3
CCL16	NCC-4, LEC, HCC-4, LMC, LCC-1,CK β 12	H	T cells, neutrophils	CCR1,2,5
CCL17	TARC	D	T cells	CCR4
CCL18	DC-CK1, PARC, MIP-4, CK β 7, DCCCK1	H	Naïve T cells	Unknown
CCL19	ELC, MIP-3 β , Exodus-3, CK β 11	H	T cells, B cells, dendritic cells, activated NK cells	CCR7
CCL20	MIP-3 α , MIP-3, LARC, exodus-1, ST38, CK β 4	D	T cells, B cells	CCR6
CCL21	SLC, 6CKine, Exodus-2, TCA4, CK β 9	D	T cells, B cells, dendritic cells, activated NK cells,	CCR7
CCL22	MDC, STCP-1, DC/B-CK	D	Macrophage progenitors	CCR4
CCL23	MIP-3, MPIF-1, CK β 8	I	T cells, eosinophils	CCR1
CCL24	MPIF-2, CK β 6, Eotaxin-2	I	Dendritic cells, osteoclasts	CCR3
CCL25	TECK, CK15	H	Effector Th2 cells	CCR9
CCL26	Eotaxin-3, IMAC, MIP-4 α , TSC-1	I	Memory T cells, B cells, immature thymocytes	CCR3
CCL27	ALP, skinkine, ILC, ESKine, PESKY, CTAK	H	Eosinophils, T cells	CCR10
CCL28	MEC, CCK1	U	CLA+ T cells	CCR10,3
C Chemokines				
XCL 1	Lymphotactin α , SCM-1 α , ATAC	D	B cells, T cells, NK cells, neutrophils	XCR1
XCL 2	Lymphotactin β , SCM-1 β	D	B cells, T cells, NK cells, neutrophils	XCR1
CX3C Chemokines				
CX3CL 1	Fractalkine, Neurotactin, ABCD-3	I	Effector T cells	CX3CR1

I, inflammatory; H, homeostatic; U, unknown; D, dual (homeostatic and inflammatory); GRO, GRO region of the CXC major gene cluster; IP10, IP10 region of the CXC major gene cluster; MCP, MCP region of the CC major gene cluster; MIP, MIP region of the CC major gene cluster. ^aAn alternatively spliced variant of CXCR3 that has been reported to mediate the ability of CXCL4, CXCL9, CXCL10, and CXCL11 to control angiogenesis.

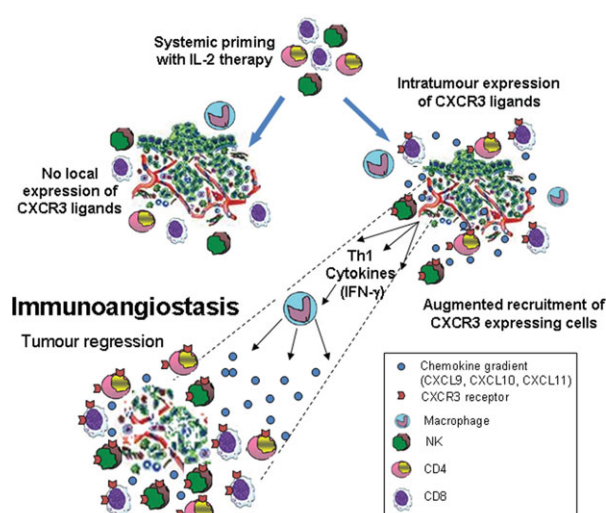


Figure 3 Priming and induction steps are necessary for the full induction of immunoangiostasis. Systemic IL-2 promotes the expression of CXCR3 on mononuclear cells, and the induction of the local expression of CXCR3 ligands within the tumour leads to the establishment of a chemotactic gradient for mononuclear cells expressing CXCR3, which concomitantly promotes angiostasis. The combined two steps of systemic priming for mononuclear cell expression of CXCR3 and local induction of CXCR3 ligand expression fully establishes immunoangiostasis.

cytokine-dependent cell-mediated immunity and concurrently inhibiting tumour-associated angiogenesis.

Among CXC chemokines, CXCL14 was initially investigated as it was down-regulated in tumour specimens of head and neck squamous cell carcinomas.⁴⁸ CXCL14 was shown to inhibit *in vitro* microvascular endothelial cell chemotaxis and *in vivo* angiogenesis stimulated by multiple angiogenic factors, including CXCL8, bFGF, and VEGF.⁵⁰ Moreover, CXCL14 expression was observed in normal and tumour prostate epithelium, and its levels were unchanged in benign prostate hypertrophy specimens.⁵¹ Interestingly, CXCL14 mRNA was found to be significantly up-regulated in localized prostate cancer and positively correlated with Gleason score.⁵¹ Studies with a model of human prostate cancer in immunodeficient mice revealed that prostate cancer cells transfected with CXCL14 were found to have a 43% reduction of tumour growth similar to controls, supporting the notion that the loss or inadequate expression of CXCL14 is associated with the transformation of normal epithelial cells to cancer cells and the promotion of a pro-angiogenic microenvironment suitable for tumour growth.

The angiostatic effects of CXCL4, CXCL9, CXCL10, and CXCL11 on human microvascular endothelial cells are apparently mediated by a unique G protein-coupled receptor, CXCR3.⁵² Recently, the existence of three different variants of CXCR3—CXCR3A, CXCR3B, and CXCR3-alt—has been demonstrated.⁵⁰ CXCR3 is preferentially expressed on activated helper T cells (Th) and plays an important role in their trafficking.^{53–55} It is still not clear whether CXCR3 ligands use their receptor on endothelium to mediate their angiostatic effect.

Human microvascular endothelial cells express the chemokine receptor CXCR3.⁵⁶ Interestingly, cultured endothelial cells are heterogeneous with respect to CXCR3 expression. Romagnani *et al.*⁵⁶ showed that these cells fail to express CXCR3 mRNA while they remain in the G0 or G1 phases of the cell cycle; on the other hand, the receptor

is induced in parallel with the cell cycle regulator cyclin A. *In vivo*, the proportion of CXCR3-positive cells is generally low and increases during inflammation, when the normally quiescent endothelial cells enter the cell cycle. This cell cycle dependence is not observed in other cell types that express the CXCR3 receptor, but causes endothelial cells to become sensitive to the antiproliferative effect of specific chemokines precisely when they are dividing or preparing to divide. Presumably, this pathway provides an additional mechanism for the physiological control of angiogenesis, which might be investigated to block tumour angiogenesis.⁶

CXC chemokines contain binding domains for both the chemokine receptor CXCR3 and GAGs. Yang and Richmond⁵⁷ demonstrated that CXCR3 receptor binding, but not GAG binding, is essential for the tumour angiostatic activity of CXCL10. A similar study on a murine model of pulmonary fibrosis showed that CXCL11 inhibits angiogenesis due to CXCR3 receptor binding, but not GAG binding.⁵⁸

4. Role of CXCR3 and its ligand in a biological axis in mediating immunoangiostasis

The role of CXCR3 in mediating angiostatic activity of CXCR3 ligands became clearer after the discovery that CXCR3 exists as two alternatively spliced forms, termed CXCR3A and CXCR3B. CXCR3A is the major chemokine receptor found on Th1 effector T cells, cytotoxic CD8 cells, activated B cells, and NK cells. The interaction between CXCR3 and CXCR3 ligands is critical for the development of anti-tumour immunity and inhibition of angiogenesis relevant to a variety of tumours.^{12,54,59–64} Indeed, while CXCR3 and CXCR3 ligands inhibit angiogenesis, CXCR3 ligands also play a critical role in orchestrating Th1 cytokine-induced cell-mediated immunity via the recruitment of mononuclear cells expressing CXCR3 (Figure 3).

CXCR3B mediates the angiostatic activity of CXCL4, CXCL9, CXCL10, and CXCL11 on human microvascular endothelial cells.^{52,65} Lasagni *et al.*⁵² showed that human microvascular endothelial cell line-1 (HMEC-1), transfected with either the known CXCR3 or CXCR3B, bound CXCL9, CXCL10, and CXCL11, whereas CXCL4 showed high affinity only for CXCR3B. Overexpression of CXCR3 induced an increase of survival, whereas overexpression of CXCR3B dramatically reduced DNA synthesis and up-regulated apoptotic HMEC-1 death through activation of distinct signal transduction pathways. Remarkably, primary cultures of human microvascular endothelial cells, whose growth is inhibited by CXCL9, CXCL10, CXCL11, and CXCL4, expressed CXCR3B but not CXCR3. The expression of the splice variant CXCR3B was significantly down-regulated by Ras, suggesting that activation of Ras plays a critical role in modulating the expression of both CXCL10 and CXCR3B.⁶⁶ This may have important consequences in the development of tumours through cancer cell proliferation.

In another study, CCL19 was demonstrated to lead to chemotaxis of dendritic and T cells to the source of the tumour antigens and to induce immune-dependent tumour reduction in a murine model of lung cancer via increases in CXCL9 and CXCL10.⁶⁷ The injection of another recombinant CC chemokine, CCL21, in tumours induced complete tumour eradication in several treated mice. The anti-tumour response was linked to CXCR3 ligands that induced

a local immunosuppressive environment via the recruitment of CXCR3 cells.⁶⁸ In immune-competent mice, intratumoural injection of CCL21 led to a significant increase in CD4 and CD8 cells as well as dendritic cells infiltrating the tumour and the draining of lymph nodes.⁶⁸ *In vivo* depletion of CXCL9, CXCL10, and interferon- γ significantly reduced the anti-tumour efficacy of CCL21. Assessment of cytokine production at the tumour site showed an interdependence of interferon- γ , CXCL9, and CXCL10. Neutralization of any one of these cytokines caused a concomitant decrease in the levels of all three cytokines. CCL21-treated tumour-bearing mice demonstrated enhanced cytolytic capacity, suggesting the generation of a systemic immune response to tumour-associated antigens. These findings are similar to the previously reported study of IL-12-mediated regression of renal cell carcinoma in a murine model, where the anti-tumour effect of IL-12 was lost when CXCR3 ligands were depleted.⁶⁹ The dual capacities of having an immunotherapeutic potential and an angiostatic effect led investigators to entertain the possibility that these small proteins can play pivotal roles in carcinogenesis and may, therefore, be potential targets for novel therapeutic approaches.

5. Conclusion

CXC chemokines are a unique cytokine family that exhibit, on the basis of structure/function and receptor binding/activation, either angiogenic or angiostatic biological activity in the regulation of angiogenesis. In addition, CXC chemokines display a key role in haematopoietic progenitor homing, B-lymphocyte development, and progenitor recruitment to sites of ischaemic tissue damage. Although the role of chemokines and immune response in angiostasis and vascular damage still needs to be further explored,⁷⁰ research in the field of immunoangiostasis has made significant achievements elucidating the combined steps of systemic priming for mononuclear cell expressing CXCR3 and local induction of CXCR3 ligand expression. Findings on the critical role of CXCR3 and CXCR3 ligands in mediating the anti-tumour effect of systemic IL-2 therapy⁶² support the notion that the systemic immunotherapy regimen could be further optimized. These results also suggest that further advances in the field of immunoangiostasis could be the right road to take for finding more efficacious therapeutic strategies by combining reduction of tumour-associated angiogenesis and augmentation of tumour-associated immunity.

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